PROFICIENCY TESTING SERVICE
AMERICAN ASSOCIATION OF BIOANALYSTS
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PARTICIPANT STATISTICS **SECOND QUADRIMESTER 2018**

GRAM STAIN

	Specimen 1		Specimen 2		Specimen 3		Specimen 4		Specimen 5		
Gram Stain	**Neg**	**Pos**	**Neg**	**Pos**	**Neg**	**Pos**	**Neg**	**Pos**	**Neg**	**Pos**	**No. of Labs**
	0	71	71	0	1	70	51	19	65	6	71
Flagging	***			***	***					***	

*Due to a lack of participant consensus, Specimen 4 was not evaluated this event. The target organism was Shigella flexneri; gram negative rods.

	Specimen 1		Specimen 2		Specimen 3		Specimen 4		Specimen 5	
Morphology	**No.**	**Flagging**	**No.**	**Flagging**	**No.**	**Flagging**	**No.**	**Flagging**	**No.**	**Flagging**
Cocci	61		0	***	56		1	***	0	
Diplococci	2	***	0		6	***	3	***	2	***
No diplococci present	0		2	***	1		1		1	
Coccobacilli	0	***	0	***	1	***	9	***	9	***
Rods (Bacilli)	0	***	62		0	***	50		52	
Streptococci	1	***	0	***	0	***	0	***	0	***
Yeast	0	***	0	***	0	***	0	***	0	***
Total Population	**64**		**64**		**64**		**64**		**64**	

Educational

	Specimen 1				Specimen 2			
Gram Stain	**Neg**	**Pos**	**Yeast**	**No pathogen found**	**Neg**	**Pos**	**Yeast**	**No pathogen found**
	0	34		0	0	28	5	0

	Specimen 1		Specimen 2	
Morphology	**No.**		**No.**	
Cocci	10		23	
Diplococci	7		0	
No diplococci present	0		0	
Coccobacilli	0		0	
Rods (Bacilli)	0		0	
Streptococci	17		1	
Yeast	0		7	
Total Population	**34**		**31**	

Image Link

Gram Stain #1 – Positive Blood Culture (Enterococcus spp.)
Clinical Presentation:
A 36-year-old male with signs of injection drug use and a three-week history of fever was admitted with cellulitis of the left arm. He had been previously treated at an outpatient clinic, but without symptomatic relief. Blood cultures were performed upon admission. A transthoracic echocardiogram (TEE) demonstrated a vegetation on the surface of the aortic valve. The Gram stain of the organism detected in all four blood culture bottles, obtained at admission, can be visualized in the attached image.

Discussion narrative:
Laboratory testing for this patient (i.e., confirmed bacteremia and echocardiographic evidence of a vegetation) was consistent with the diagnosis of infective endocarditis (Duke clinical criteria for infective endocarditis). The most common etiological agents of endocarditis (i.e., specifically, for injection drug users) are Staphylococcus aureus, viridans group streptococcus, Enterococcus spp., Candida albicans, and Gram-negative rods (e.g., Pseudomonas aeruginosa). Gram stain of the organism detected in both sets of the blood cultures obtained at admission showed Gram-positive cocci arranged in pairs and shorts chains, and through standard subculture techniques, the patient was determined to be infected with an organism belonging to the genus Enterococcus.

Enteroccocci are bacteria normally present in the gastrointestinal and female genital tracts, but can also colonize the skin, as well. Thus, this organism can get into open wounds or skin ulcers and cause infection, as in the present community-acquired infection. However, Enterococci is more often seen, clinically, in the context of hospital-acquired (nosocomial) infections (e.g., bacteremia, urinary tract infection, peritonitis) as Enterococcal infections are fairly common in patients with intravascular or urinary catheters, and in patients who have been hospitalized and/or given empiric/broad-spectrum antibiotics. In this case, a previously damaged heart valve was most likely colonized through transient bacteremia brought about through the patient's intravenous drug use. The antibiotics prescribed at the outpatient clinic were ineffective due to the inherent resistance of this organism to antibiotics, as well as the poor penetration of antibiotic agents into such thrombotic lesions, in general. Consequently, a cell wall agent (e.g., ampicillin or vancomycin) is typically given in combination with gentamicin to treat enterococcal endocarditis.

Enterococci grow readily on nonselective media used routinely in clinical microbiology laboratories (e.g., blood and chocolate agar) and can be α, β or non-hemolytic on blood agar. While these organisms resemble S. pneumoniae on Gram-stained specimens, the organisms can be readily differentiated through common phenotypic testing. For example, enterococci are resistant to optochin; whereas, pneumococcus is susceptible. In addition, enterococci are not dissolved when exposed to bile (S. pneumoniae is dissolved or bile soluble). Enterococci are also positive for both the bile esculin hydrolysis (i.e., hydrolyzes esculin in the presence of 40% bile) and PYR (Pyrrolidonyl Aminopeptidase) tests, both of which are negative for streptococci, in general (exception: S. pyogenes is PYR+). Speciation within the Enterococcus genus typically requires additional phenotypic, proteomic, or nucleic acid-based testing, all of which represent robust alternatives to conventional testing for the identification of enterococcal species in routine diagnostic laboratories.

Image Link

Gram Stain #2 - Positive Blood Culture (Staphylococcus epidermidis)
Clinical Presentation:
A 56-year-old woman, with a medical history of type 2 diabetes and hypertension was otherwise healthy until three days prior to admission when she developed fever, chills and a productive cough. Over the last two of days, leading up to admission, she complained of increasing dyspnea and chest pain. A chest radiograph confirmed right and left lower lobe infiltrates. The culture of the sputum grew 4+ Streptococcus pneumoniae (and respiratory flora). Of two sets of blood cultures drawn prior to the administration of antibiotics, one aerobic bottle was positive for the Gram-stained organism in the attached photomicrograph.

Discussion narrative:
With the information provided in the clinical presentation, the patient's blood culture isolate would be most suspicious for a blood culture contaminant, given the Gram stain result (i.e., Gram-positive cocci in clusters; inconsistent with S. pneumoniae Gram stain morphology) along with the fact that only one blood culture bottle was positive among the two sets drawn upon admission. In fact, the biochemical testing revealed an organism that was positive for catalase, negative for coagulase, and later confirmed to be Staphylococcus epidermidis, and subsequently reported as coagulase-negative staphylococci (CoNS). Therefore, the preliminary interpretation (suggested above) of the blood culture result is supported by the patient's clinical picture, which is most consistent with community-acquired pneumonia due to S. pneumoniae. In fact, approximately two-thirds of patients with pneumococcal pneumonia do not have positive blood cultures for the pneumococcus.

CoNS are ubiquitous, and part of the normal human skin and mucosal microbiota. These organisms are found in small numbers in cultures of skin and soft tissues and in this setting frequently noncontributory to the disease process. While CoNS lack many of the virulence factors associated with Staphylococcus aureus, this group of organisms are still considered opportunistic pathogens. Accordingly, CoNS can readily form biofilms on solid surfaces, and therefore, recognized as important causative agents of infections involving a variety of catheters and prosthetic devices (e.g., drive lines for cardiac assistance devices, intravascular catheters, prosthetic joints, etc.). These infections can be due to several of the more than 30 species of CoNS that have been described. The species most commonly associated with these infections is Staphylococcus epidermidis, which is well recognized for its ability to grow as biofilms on solid surfaces. S. epidermidis are facultative anaerobes that form greyish-white, raised, smooth colonies (1–2mm in diameter) after overnight incubation, and is non-hemolytic on blood agar. They are positive for the salt tolerance test (i.e., grow well at NaCl concentrations up to 7.5%), catalase- and urease-positive and exhibit a weak positive reaction for the nitrate reduction test. They are negative for coagulase, oxidase and gelatin hydrolysis tests. S. epidermidis is also sensitive to novobiocin, and this test distinguishes it from Staphylococcus saprophyticus (i.e., uropathogen in sexually active women), which is coagulase-negative, as well, but novobiocin resistant.

Importantly, and as seen in this case, the significance of the recovery of CoNS from a patient's blood culture must be determined in the clinical context, as such results can otherwise lead to the over-prescription of antibiotics and potentiating antimicrobial resistance and risks for adverse drug reactions. In addition, contaminated blood cultures can be expensive, as patients with false-positive blood cultures stay in the hospital for an additional day and, typically, have additional (unnecessary) diagnostic procedures performed, as well. In addition to CoNS, other organisms that are often considered contaminants include skin microbiota such as Micrococcus spp., diptheroids, Bacillus spp., Propionibacterium acnes, and viridans group streptococci. Please note that several participants incorrectly identified these organisms as yeast. Be sure to use the micrometer tool when you view the image. *Staphylococcus epidermidis* are 1-2 microns in diameter. A typical *Candida albicans* yeast form is 10-12 microns.